Update in Investigation of SLE

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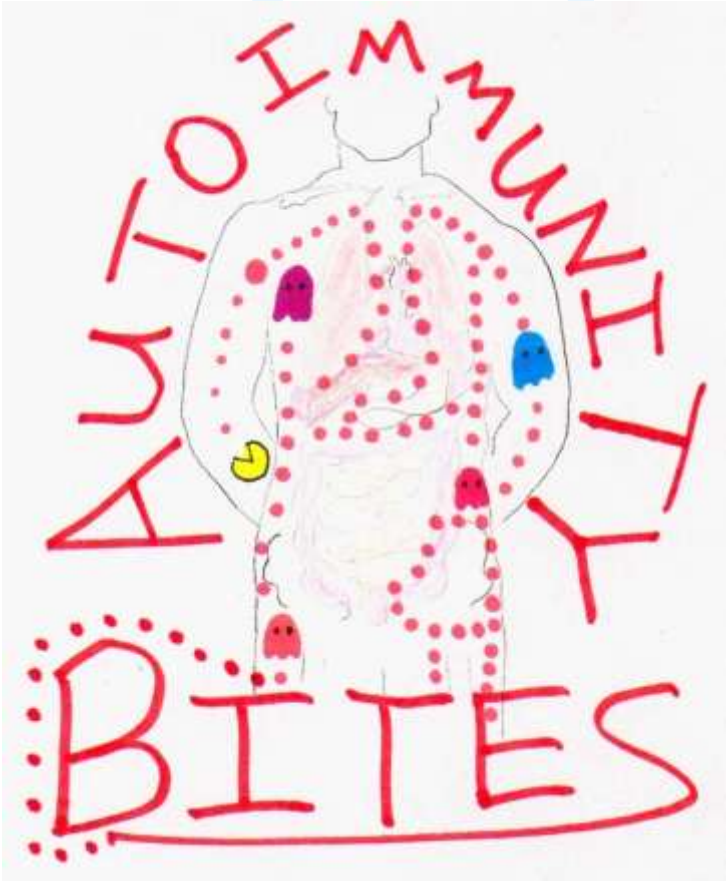
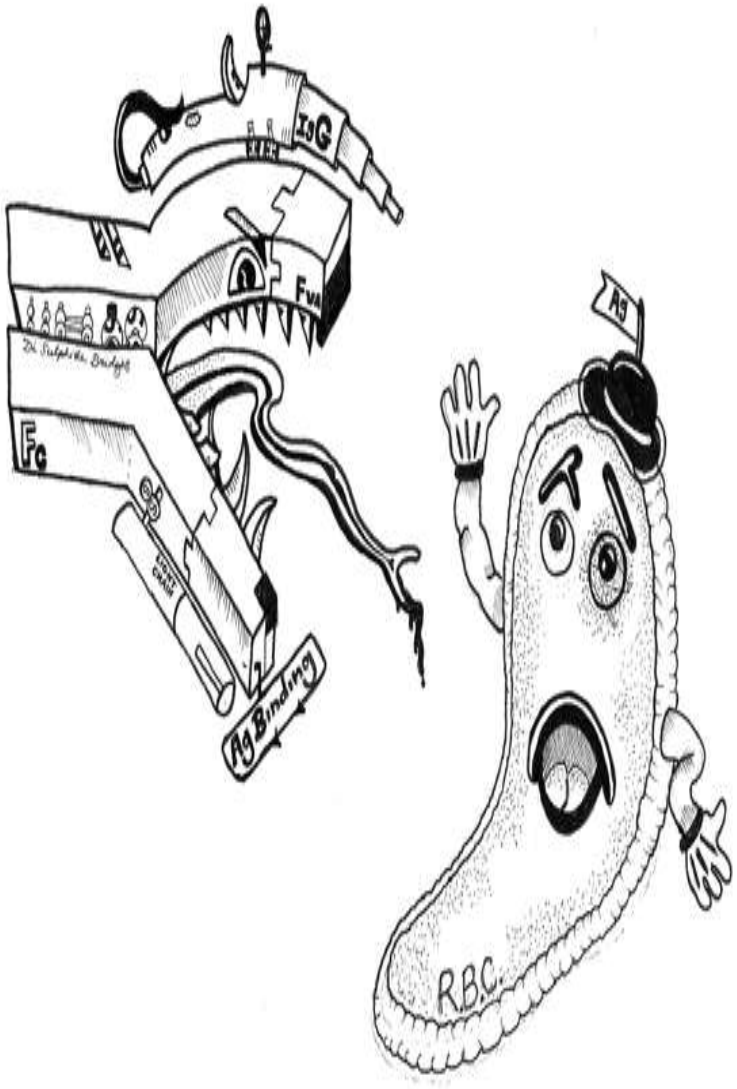


SLE is the most clinically and serologically diverse AID , with more than 100 auto-abs found in patients and disease spectra ranging from subtle symptoms to life-threatening multi-organ failure

The hallmark characteristics production of auto-abs, deposition of IC, and excessive complement activation (consequences of immune dysregulation)

Auto-abs are directed against intranuclear nucleic acids, proteins and nucleoprotein complexes



steps in SLE development

genetic predisposition

gender

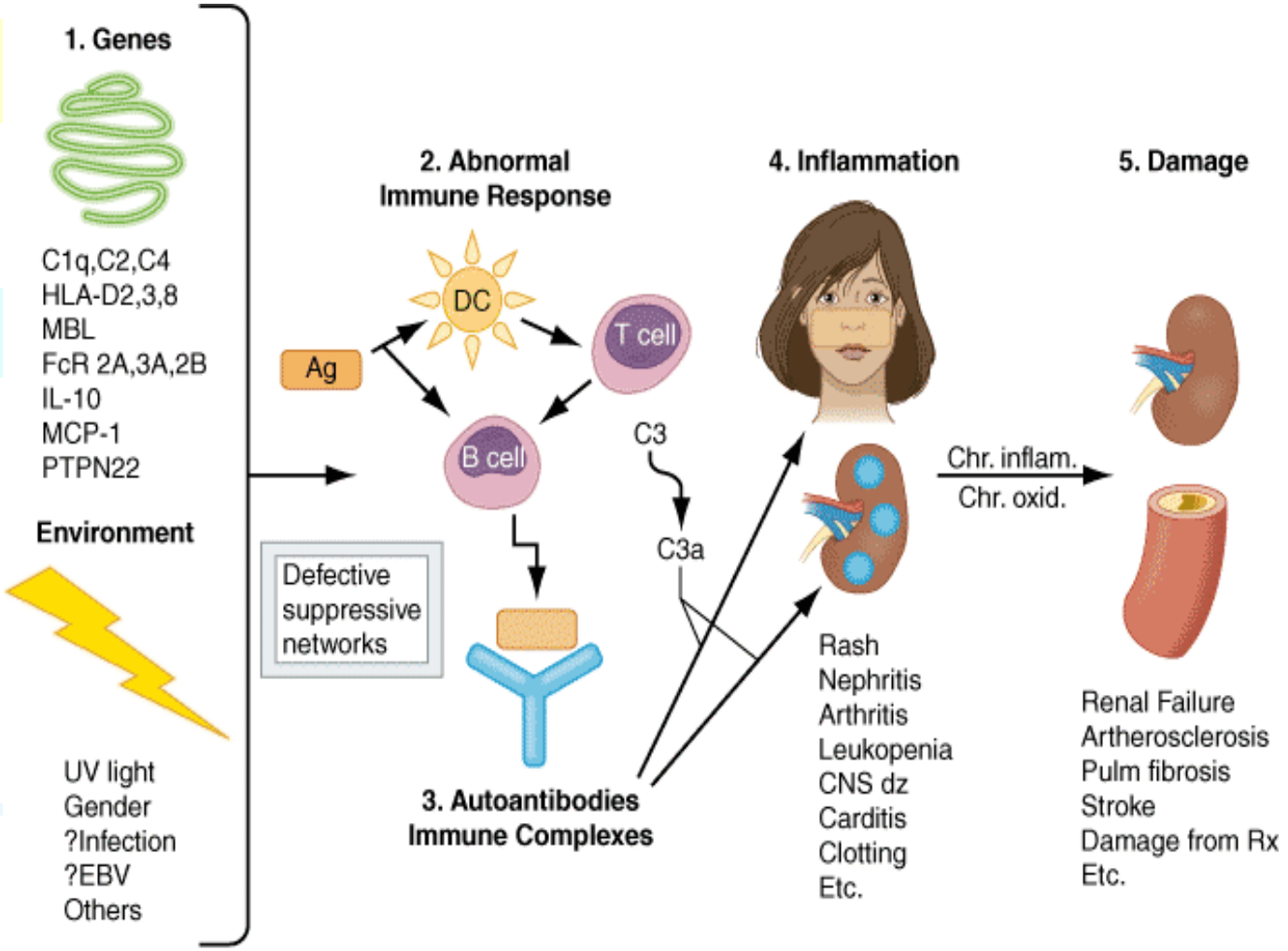
environmental stimuli

Abnormal IR

autoantibodies +IC

Clinical manifestation

chronic inflammation and oxidative damage

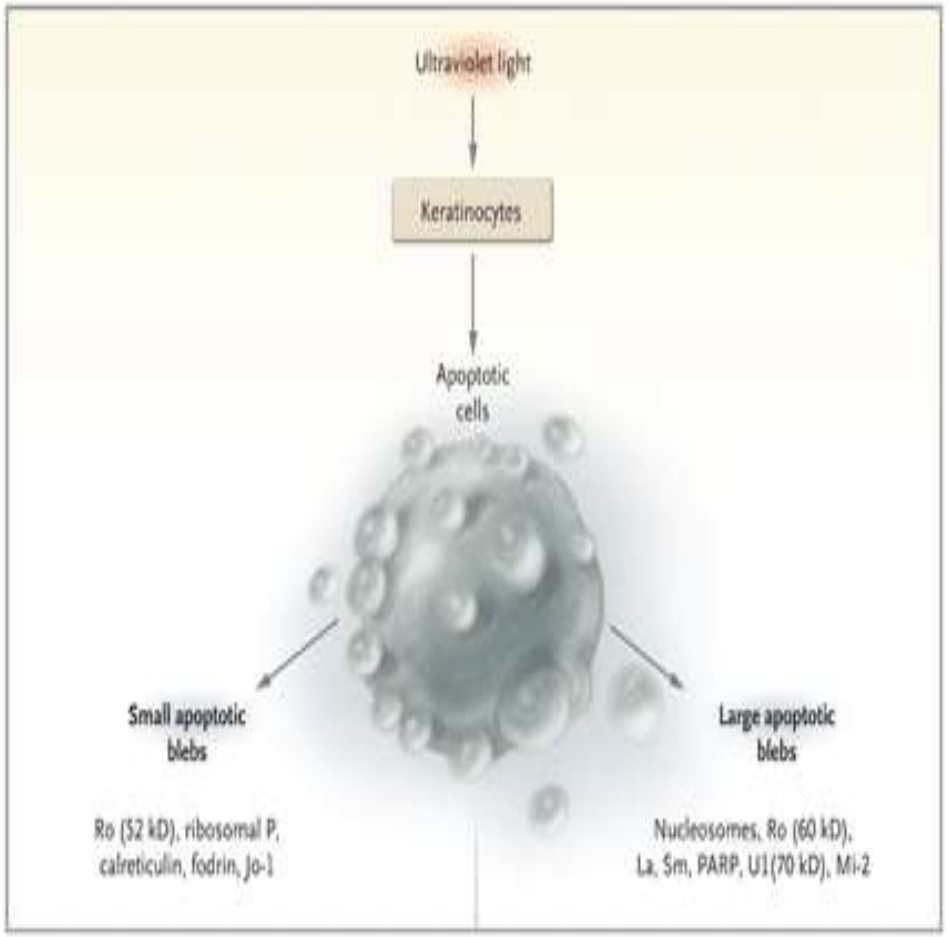


Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

ANTIGEN In SLE

- **Foreign antigen**: molecular mimicry following activation by microbial antigen can initiate autoreactivity

Self antigen: nucleosomes is the cellular debris released as a result of apoptosis. During apoptosis, blebs of cellular material form on the surface of the dying cell. Antigens that are normally buried within the cells are exposed on the surface of these blebs , and they may trigger an immune response. These exposed antigens include nucleosomes, Ro 62, Ro 50, La, and anionic phospholipids.



Pathogenesis of SLE

Autoantigen

TLR + PDC

IFN-α

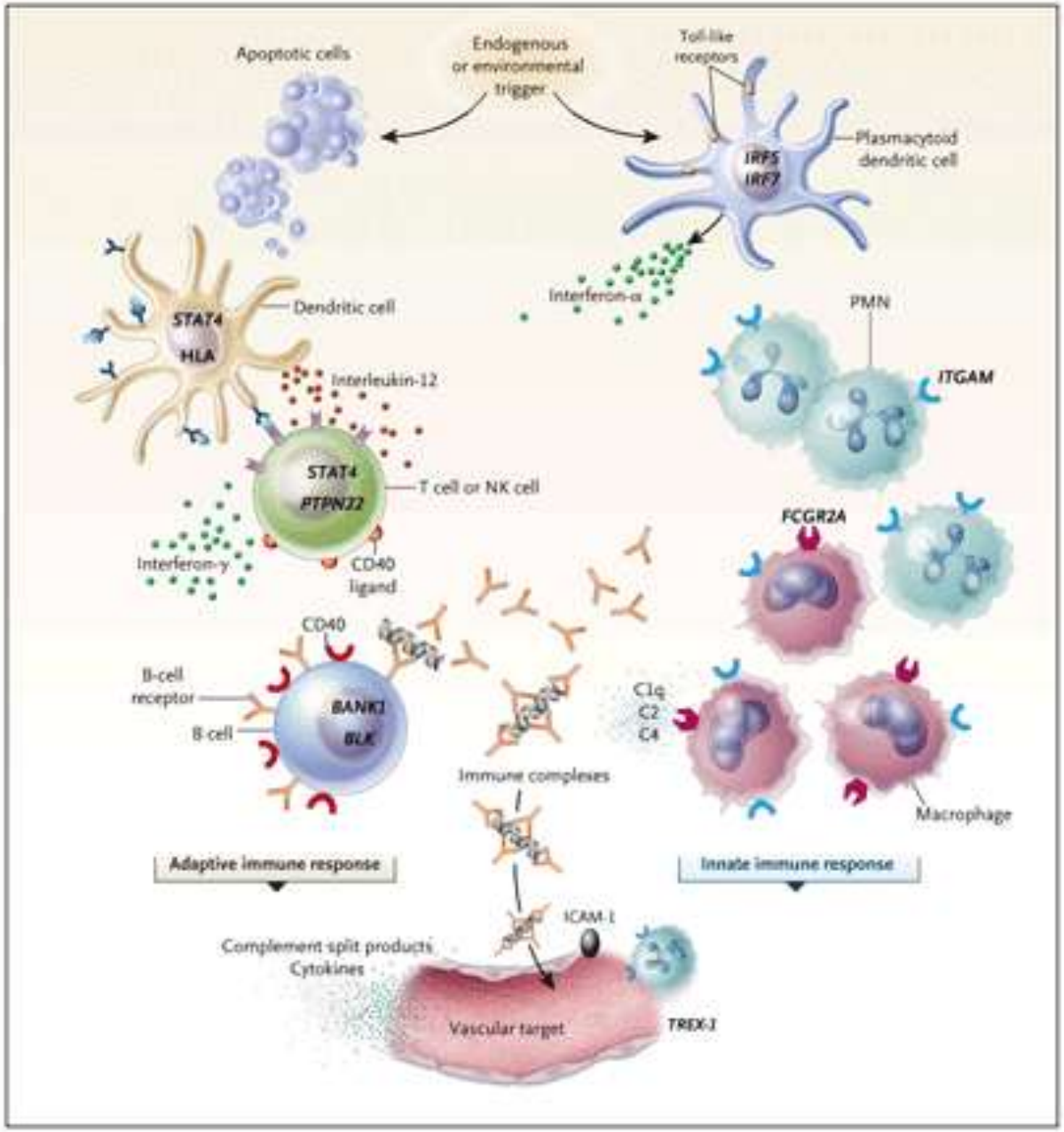
DC, T, NK

B-cells

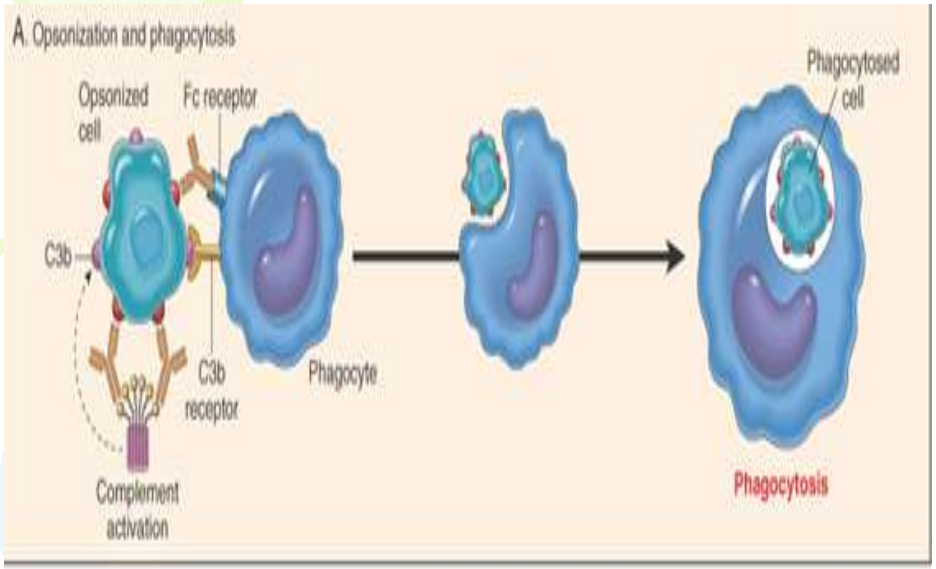
IC

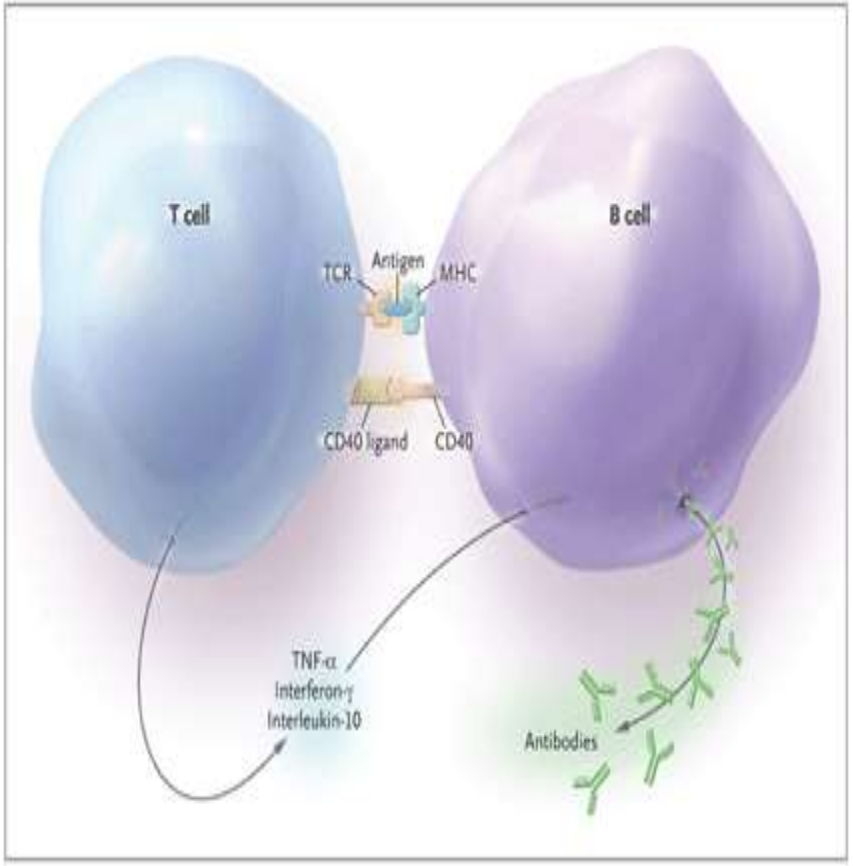
PMN + Macrophages

Crow NEJM 2008 :358(9):956-961



Tissue Injury in SLE

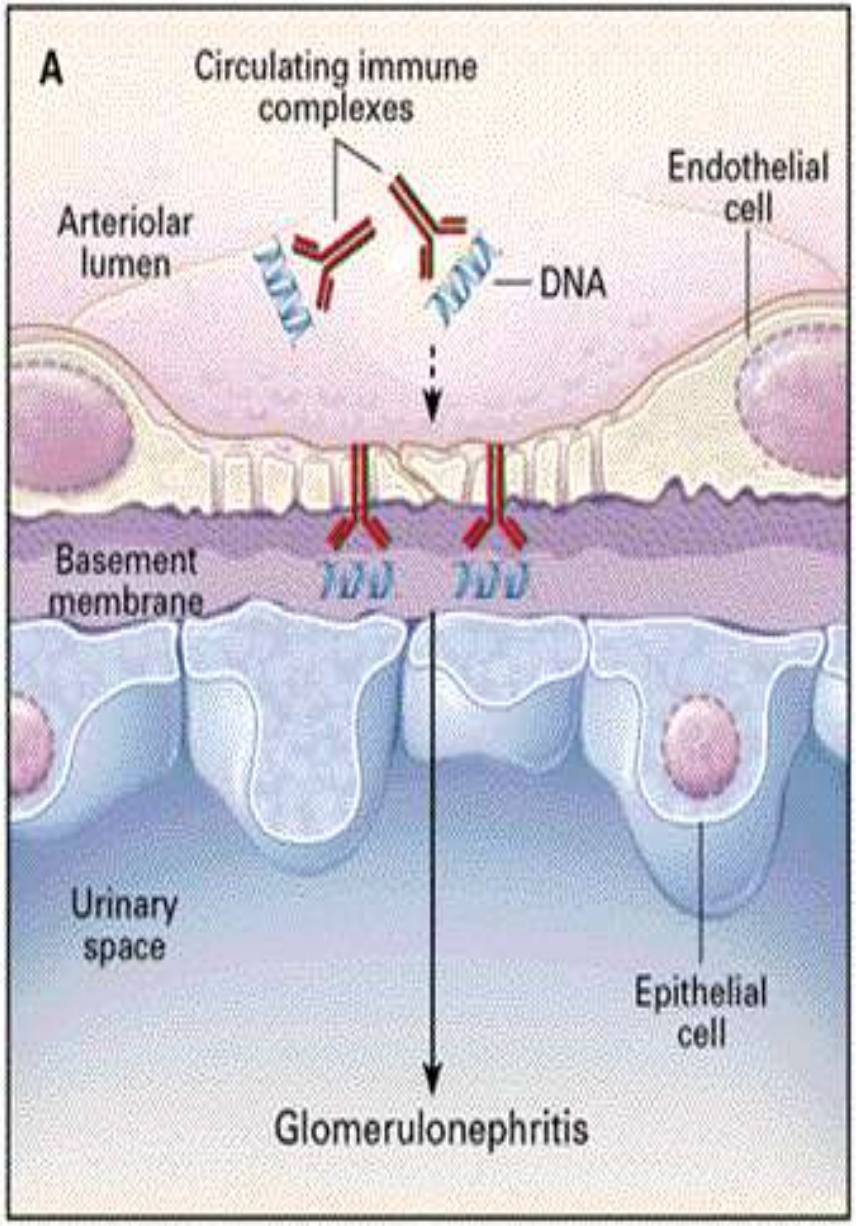




anti–ds- DNA autoantibodies bind to nucleosomes, these ab–Ag complexes settle in the renal glomerular basement membrane. These immune complexes activate complement, which initiates the glomerulonephritis

Ds-DNA, nucleosome abs cross-react with proteins in the kidney; thus, they have a direct pathogenic effect on renal cells.

Polyreactivity: the same ab can bind to ags with different structures because they have similar surface shapes (shared epitopes) or areas of similar charge. α-actinin



1- Environmental Influences

2-Female Hormones and Sex

3-Epigenetic Regulation of Gene Expression

4-Abnormalities in immune cells and cytokines.

5-Role of Innate immunity

6-Immune deficiency and autoimmunity

7- Genetic susceptibility.

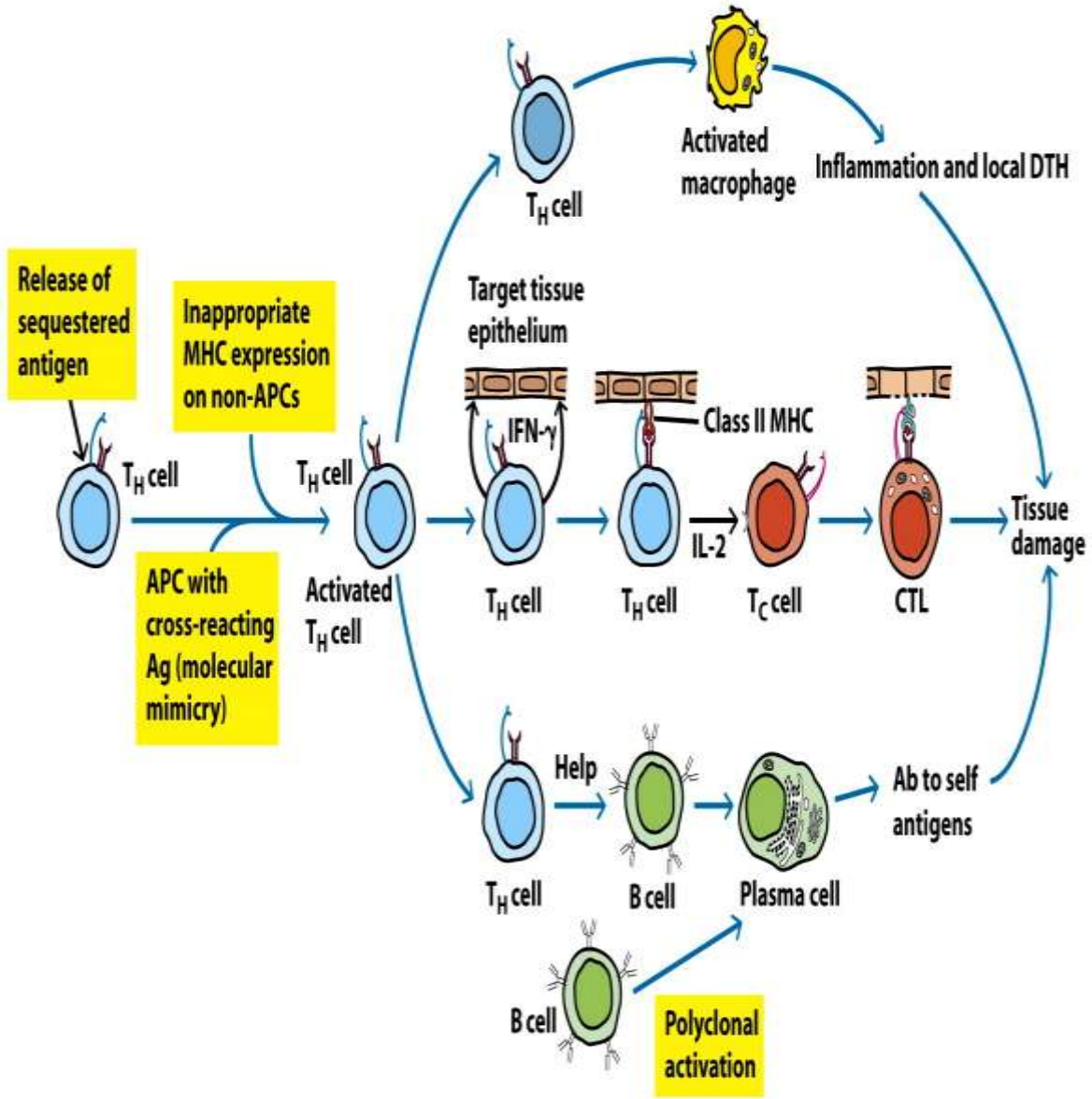


Figure 16-12
Kuby IMMUNOLOGY, Sixth Edition

TABLE 16-3	Molecular mimicry between proteins of infectious organisms and human host proteins
Protein*	**Sequence†**
Human cytomegalovirus IE2	79 P D P L G R P D E D
HLA-DR molecule	60 V T E L G R P D A E
Poliovirus VP2	70 S T T K E S R G T T
Acetylcholine receptor	176 T V I K E S R G T K
Rabies virus glycoprotein	147 T K E S L V I I S
Insulin receptor	764 N K E S L V I S E
Klebsiella pneumoniae nitrogenase	186 S R Q T D R E D E
HLA-B27 molecule	70 K A Q T D R E D L
Adenovirus 12 E1B	384 L R R G M F R P S Q C N
α-Gliadin	206 L G Q G S F R P S Q Q N
Human immunodeficiency virus p24	160 G V E T T T P S
Human IgG constant region	466 G V E T T T P S
Measles virus P3	13 L E C I R A L K
Corticotropin	18 L E C I R A C K
Measles virus P3	31 E I S D N L G Q E
Myelin basic protein	61 E I S F K L G Q E

*In each pair, the human protein is listed second. The proteins in each pair have been shown to exhibit immunologic cross-reactivity.

†Amino acids are indicated by a single-letter code. Identical residues are shown in blue. Numbers indicate amino acid position in the intact protein.

SOURCE: Adapted from M. B. A. Oldstone, 1987, *Cell* **50**:819.

Table 16-3
Kuby IMMUNOLOGY, Sixth Edition

Lysine -Glutamic acid -Serine -Arginine -Glycine -Threonine

hogen and Auto-Immune D

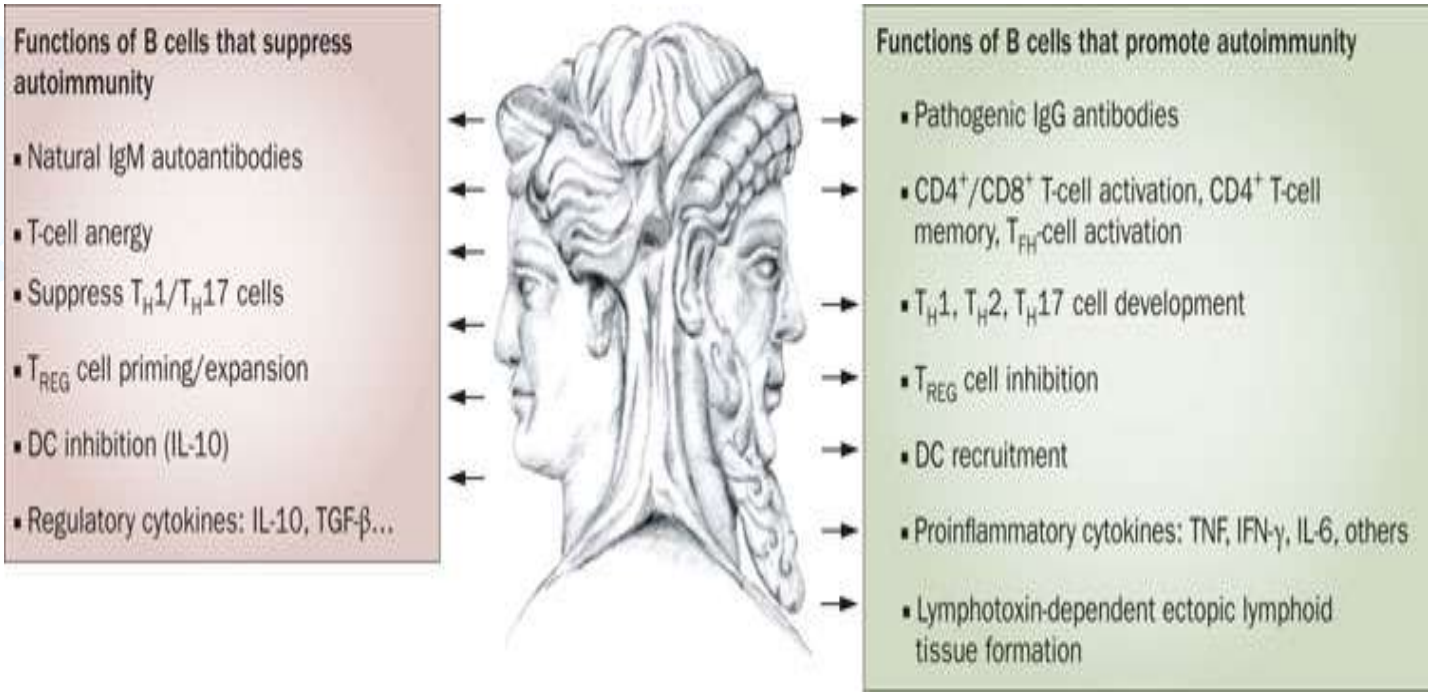
Clinical Reviews in Allergy and Immunology

ISSN 1080–0549/07/32(1):111–118/$30.00
ISSN Online 1559–0267

	Pathogens	References
	Trypanosoma cruzi	3,4,8,9
	Streptoccocus pyogenes	10–12
nts	Herpes virus, *Hemophilus influenzae*	3,13,14
	Corona, measles, mumps, EBV, herpes	4,15–17
	Campylobacter jejuni	18,19
	Coxsackievirus B, Rotaviruses, Herpes, hepatitis C, rhino-, hanta retroviral	18,19
	Klebsiella pneumoniae, chlamydia	4,22,23
	Hemophilus influenza, *Neisseria gonorea*, Tetanus toxin, CMV	24,25
	EBVpneumococcal polysaccharide	26–29

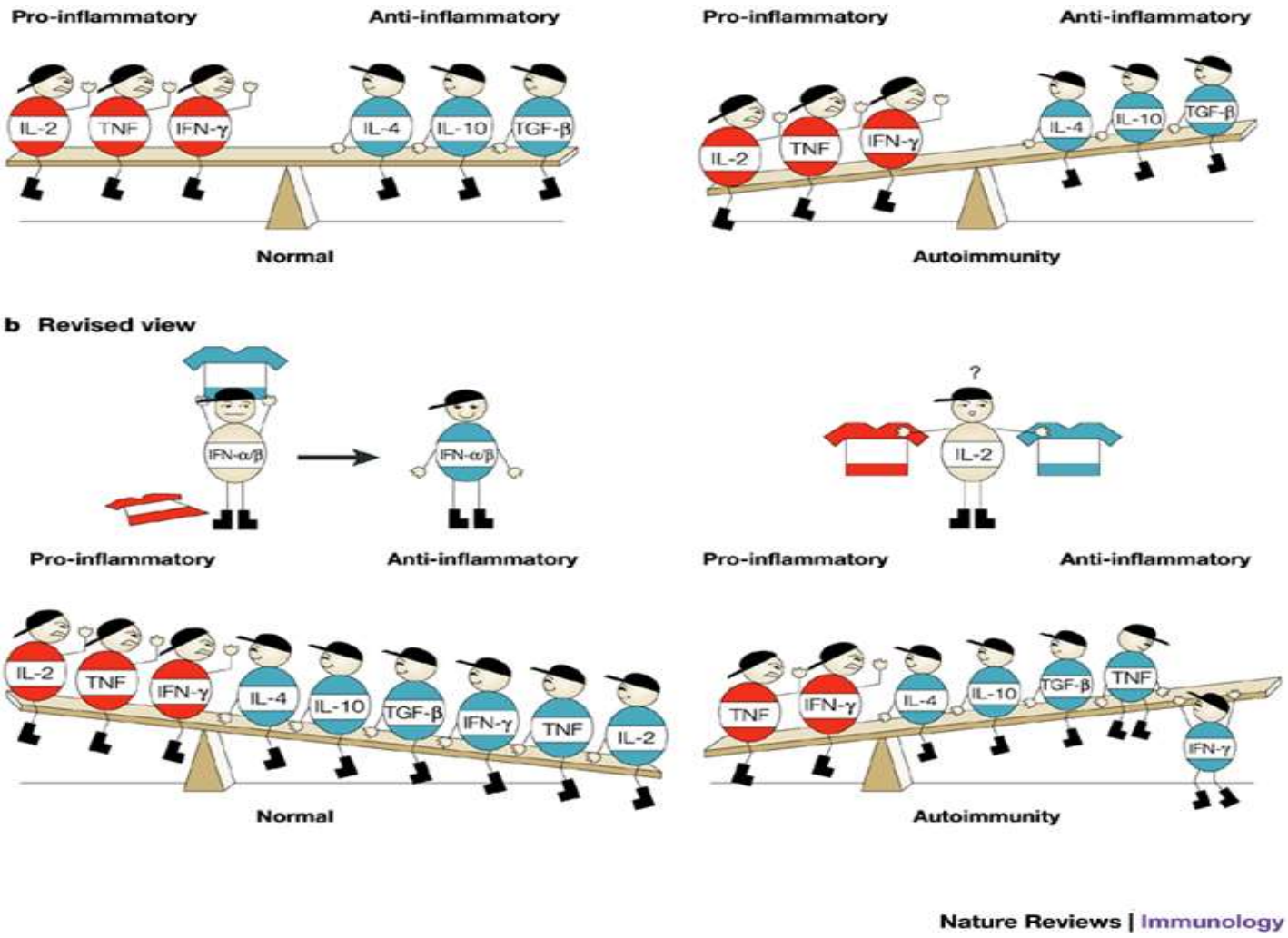
Abnormalities in immune cells

*Abnormal T cell activation and regulation
*Increased longevity of autoreactive T cells
*Increased interleukin-17
*Abnormal increased expression of CD44.
*Deficient production of interleukin-2
*A high percentage of CD4+ T cells and DN-T
*Hyperactive B lymphocytes
*Defect in Breg (IL-10 , TGFB1)



Sanz, I. & Lee, F. E. -H. (2010) B cells as therapeutic targets in SLE *Nat. Rev. Rheumatol.*

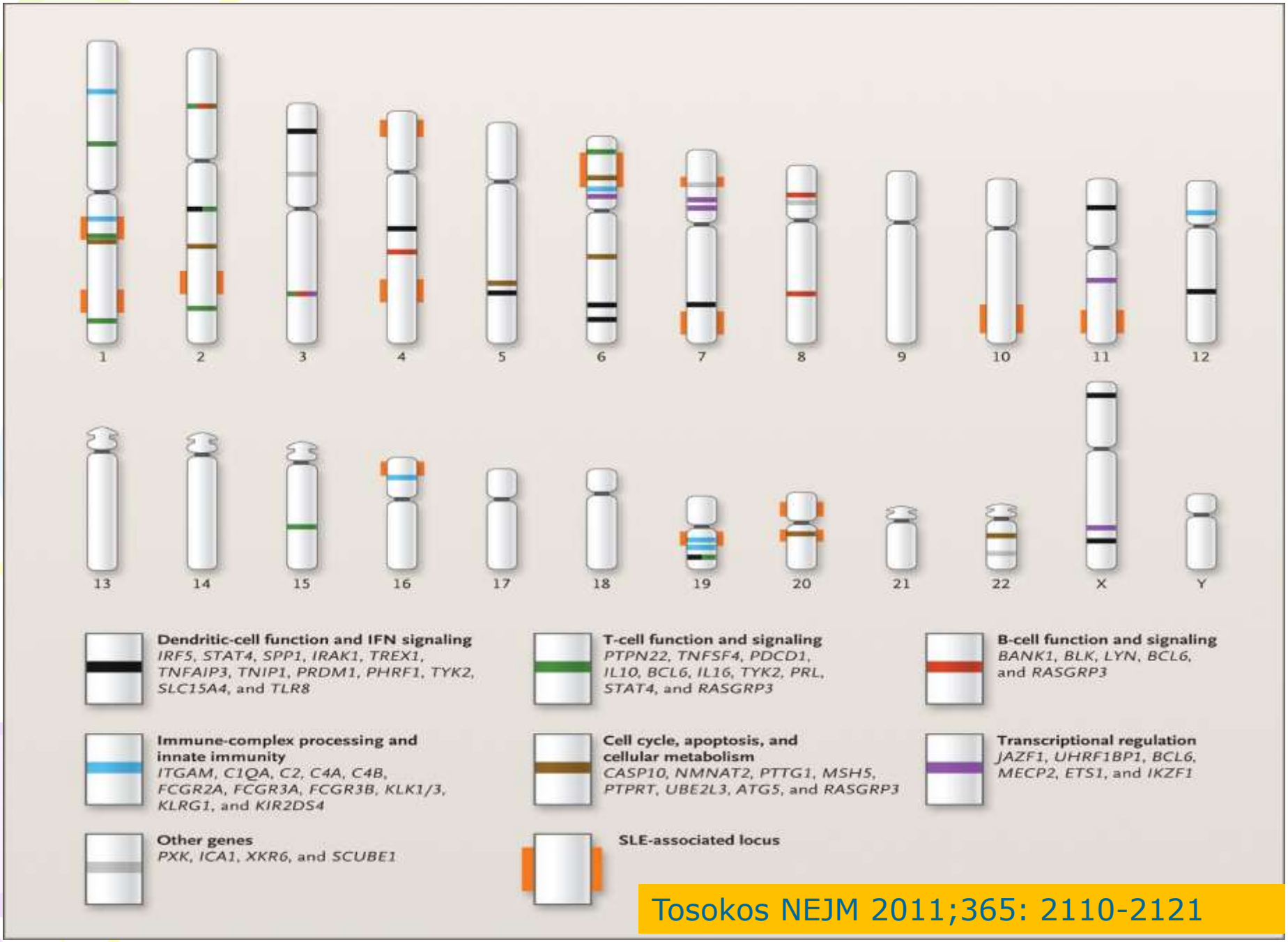
cytokine



*High type I interferons *High TNF-α, especially in renal tissue

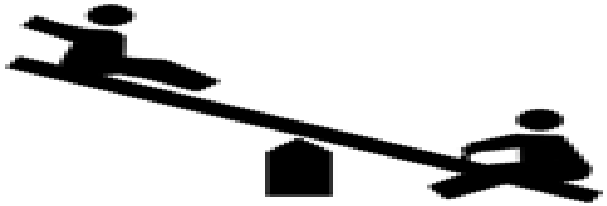
*High type II interferons (IFN-γ) * High IL-6, IL-17

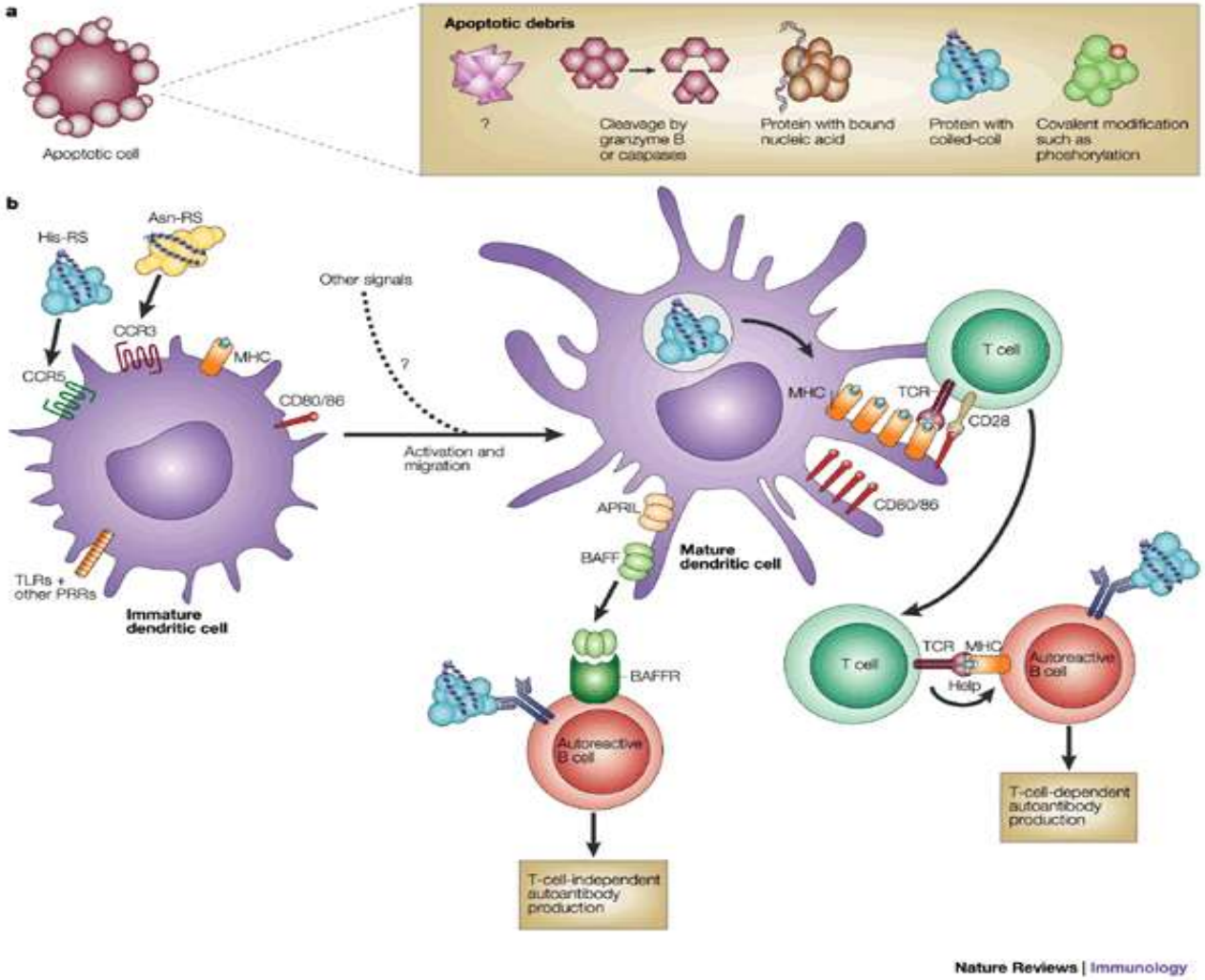
*Low IL-2 ,IL-10 *Low TGF-β



Tosokos NEJM 2011;365: 2110-2121

The Complement and SLE

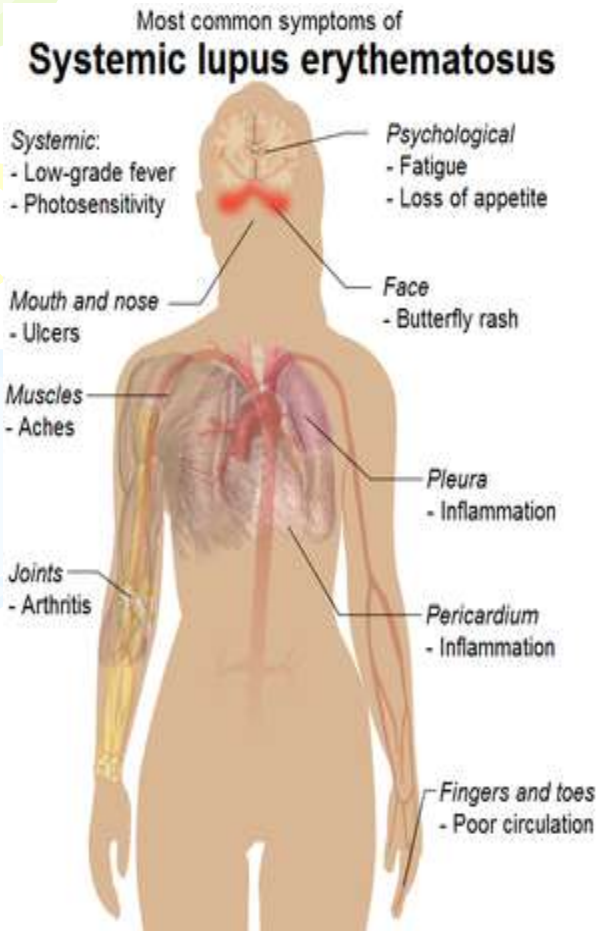




I- Clinical features

Characteristic: ACR

Diagnosis



Fatigue , Muscle pains
Fever , Loss of appetite
Weight loss

S	Serositis	heart, lung, peritoneum
O	Oral ulcers	painless esp palate
A	Arthritis	non-erosive
P	Photosensitivity	
B	Blood disorders	↓RBC (Coombs +), PLT, WCC, Lymphocytes
R	Renal involvement	proteinuria /± casts
A	ANA	titer > 1:160
I	Immunologic phenomena	anti-dsDNA Ab, anti-Sm Ab, antiphospholipid Ab, false WR +
N	Neurological disorders	seizures/ psychosis
M	Malar rash	cheeks + nasal bridge
D	Discoid rash	rimmed with scaling, follicular plugging

Diagnosis

II-General tests of inflammation

•Initial testing – not disease-specific but may be helpful in determining organ involvement

- **CBC – anemia, thrombocytopenia, leukopenia**
- **Urinalysis – hematuria, proteinuria, cast (renal disease)**
- **Liver transaminases – ± elevated (acute phase response)**
- **BUN/creatinine – may be elevated; indicates renal disease**
- **ESR: Elevated (active , infection)**
- **CRP – elevated (Infection, CV disease)**
- **Hypergammaglobulinemia (immune activation)**
- **ANCA – rule out vasculitis**
- **Complement 3 and 4 (C3, C4) – decreased levels (active,GN)**
- Cardiovascular risk screening :fifty times more likely to develop cardiovascular disease , blood glucose and lipid profile

III- Autoantibodies

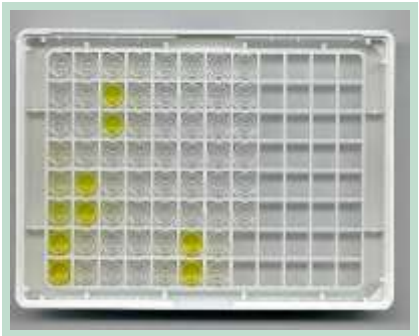
Diagnosis

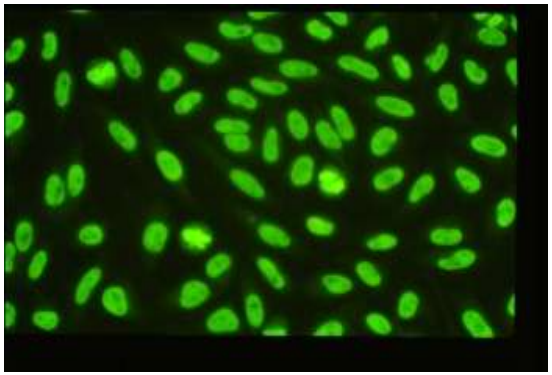
No test for ANA and for specific autoantibodies to nuclear antigens should be performed without a clinical evaluation that leads to a presumptive diagnosis

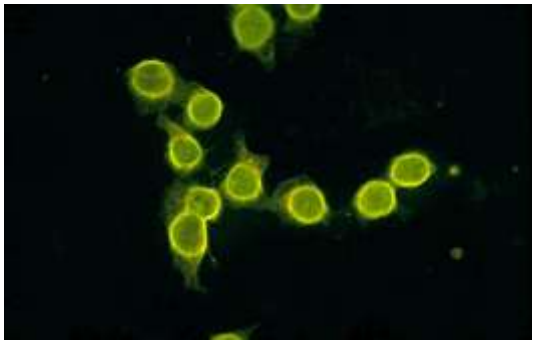
Kavanaugh et al. *Guidelines for clinical use of the ANA test and tests for specific autoantibodies to nuclear antigens.* Arch Pathol Lab Med. 2000;124:71-81.

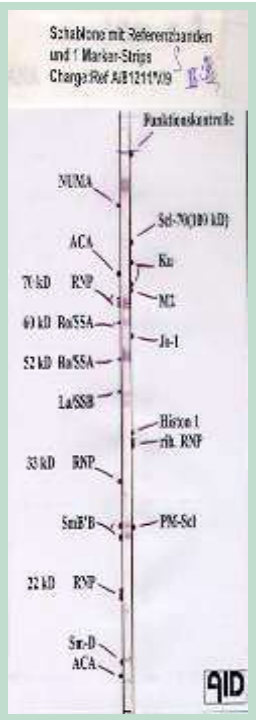
low titers of ANA

ANA are not specific









Titer rises with age

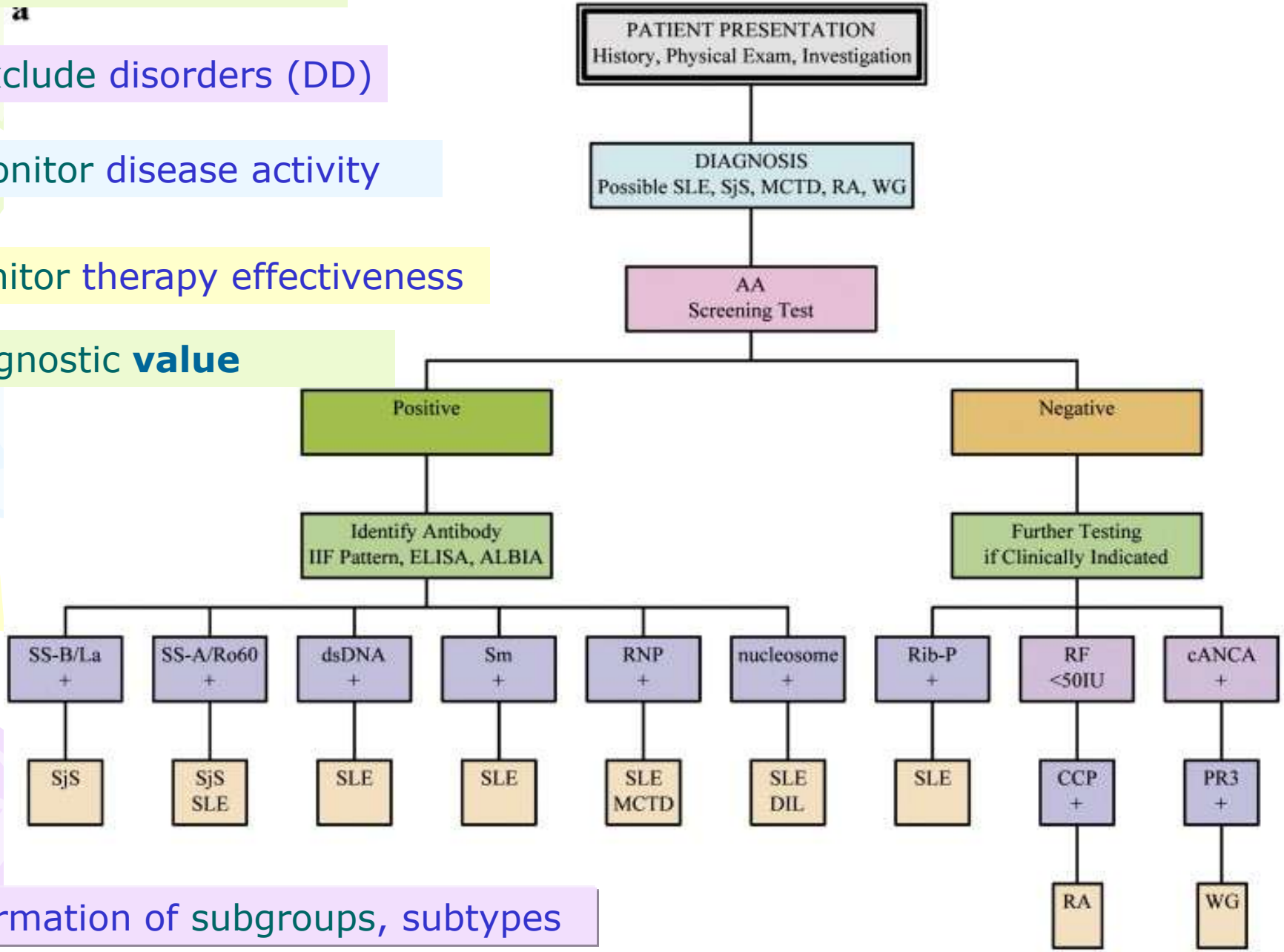
Verify a diagnosis

Exclude disorders (DD)

Monitor disease activity

Monitor therapy effectiveness

Prognostic **value**



formation of subgroups, subtypes

ANA

ANA by IIF useful screening , one of ACR criteria

Most people with SLE have ANAs (95%) , Most patients with ANAs do not have SLE

+ve ANAs are common 5% health , 30% unwell elderly, low titer

Not specific (other autoimmune , chronic infections, post acute viral infections)

High titer 1/160 or more / Rim – homogenous/ IgG

No correlation with disease activity

ANA negative SLE (technical artifact or subgroup of SLE)
Most are positive in DNA or ENA assays

Recommendation

ANA testing indicated
Clinical evidence CTD

ANA testing not indicated
-No significant clinical suspicion of SLE
-To evaluate fatigue, back pain or unless accompanied by one or more of the clinical features SLE
- To confirming a diagnosis of RA or osteoarthritis.

-ANA testing should usually be ordered only once.
-Positive ANA tests :
*do not need to be repeated (No correlation with activity)
*must be confirmed by specific autoab(ds-DNA)

Negative tests : rarely need to be repeated. If there is a strong suspicion of an evolving CTD or a change in the patient's illness suggesting the diagnosis should be revised, repeat testing may be indicated.

-ve ANA + ds-DNA +ve
Anti-Ro60 / Smith

d S- D N A

Highly specific for lupus (70% of patients)

systemic lupus and nephritis, but not subacute cutaneous lupus or discoid lupus.

Specific assays should be used for diagnosis (IIF) , whereas sensitive assays might be more useful for monitoring (ELISA).

Be sure :it is ds-DNA (not ss-DNA) , and IgG or IgA (Not IgM)

Rise in active disease and in the evolution of lupus nephritis in most patients , therefore , regular sampling every 6-8 weeks

autoimmune hepatitis, and infections including syphilis, parasitic infections and bacterial endocarditis.

Negative ds-DNA SLE : early in disease, after treatment, or when the patient is in clinical remission
60% -ve ds-DNA -------- antinucleosome abs

A-HJST ONe

Characteristic for Drug induced antibodies

50–80% of SLE have IgG and IgM antihistone antibodies

proteinuria, glomerulonephritis, and disease activity

A-SM/RNP

High titer anti-Sm constitutes an ACR criterion for SLE and is highly SLE-specific. (30%)

are not useful for monitoring disease activity. LN,CNS

Anti-Sm antibodies are rarely found without anti-RNP. Anti-RNP is more common (40%) and less specific for SLE

Anti-RNP abs are not strongly associated with specific clinical features of SLE, outside MCTD

R O / La

IgG are found in SLE and Sjogren's syndrome.

Anti-Ro60 (SLE) , Ro60 and Ro52 (Sjogren's syndrome)

May the titer reflect SLE activity

not specific SLE, but very useful with negative ANA

-ve ANA + -ve Ro60 = safe to rule out SLE

anti-Ro is associated with cutaneous involvement in subacute cutaneous lupus and with CHB

Anti-nucleosome antibodies

-In 70-100 % of SLE ,High specificity up to 97 %
-Lupus nephritis
-ve ds-DNA SLE
-strong correlation with SLE disease activity

Anti-HMGB1

-Cutaneous lupus 35%
Correlate with disease activity / Correlate with ds-DNA

Ribosomal P antibodies

associated with neuropsychiatric SLE, but their predictive value is uncertain and controversial. Titre rise in active SLE.

Neuronal / NR2 antibodies

Neuropsychiatric SLE, but their predictive value is uncertain and controversial.

Anti-C1q

up 50% and are associated with renal involvement.
Correlate with LN , LN activity, ds-DNA

Anti-CRP

Correlate with course of SLE.

Antiphospholipid antibodies

ACL of all isotypes are seen in 16–60% of patients with SLE

IgG ACAs are a risk factor for thrombosis and the APL syndrome,

IgG anti-ß2 glycoprotein 1 antibodies are more closely associated with thrombosis in the primary antiphospholipid syndrome and SLE, and approximately 25% of SLE patients may be positive.

ACL abs (IgG or IgM) Two or more occasions, at least 12 wk apart
LA screening and confirmatory testing on at least two separate occasions more than 6 weeks apart
Anti-β2-glycoprotein : Two or more occasions, at least 12 wk apart

Cell membrane associated DNA (cmDNA)

Anti-endothelial cell abs

Proliferating cell nuclear antigen(PCNA)

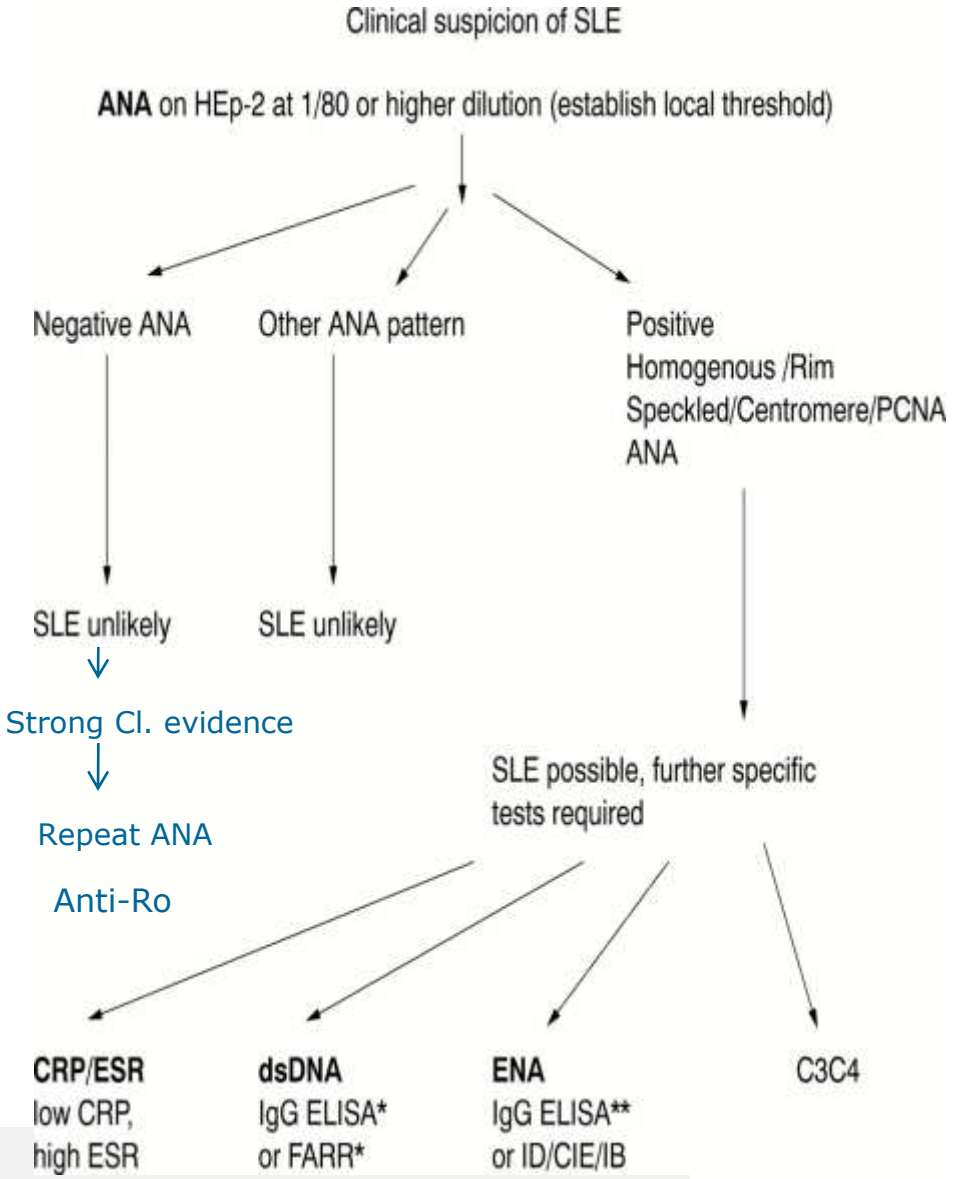
Name	%	Clinical significance
ANA	95	Best screening test; repeated negative tests make SLE unlikely
Anti-ds-DNA	70	Disease specific Lupus nephritis correlate with disease activity
Smith	30	Disease specific
RNP	40	Not specific /MCTD-overlap SLE
RO /SSA	30	Not specific , SS Neonatal / cutaneous lupus SLE elderly / dec risk of LN
La/SSB	10	Always +Ro /If +ve LN risk low
Histone	70	Drug-induced lupus
Phospholipid	50	APLS
Ribosomal	20	CNS lupus
Nucleosome	70-100	Specific, LN, + dse activity, -ve ds-DNA
Platelet	30	Thrombocytpenia
WBCs/ RBCs	70	Leucopenia / lympopenia/ anemia
C1q	47	Lupus nephritis
HMGB1		Cutaneous lupus

Diagnosis

-ANA IIF is an effective screening assay
-High titer ≥1/160
-Peripheral /homogonous pattern (detect most autoantibodies).

ANA positive more specific assays for the diagnosis of SLE. A combination of ENA (Ro/La/Sm/RNP) and ds-DNA assays will detect most patients with SLE

-ANA negative samples
--+high clinical suspicion/ change clinical -------- Repeat ANA
- +ve ds-DNA ---------- Ro



Monitoring

-ELISA (Quantitative)
- No ANA testing
- anti-dsDNA, ncleosome , Ro , C1q , Apl, C3, C4, CRP, ESR

Lupus Biomarker

A biomarker can be defined as a genetic, biological, biochemical, or molecular event whose alterations correlate with disease pathogenesis and/or manifestations and can be evaluated qualitatively and/or quantitatively in laboratories

Category	Marker
Disease susceptibility	• Complement (C1q, C2 and C4) deficiency • FcγRIIa, FcγRIIb, FcγRIIIa polymorphism • MBL polymorphisms • MCH alleles (DRB1, A1 and B8) • IL-10, IL-6 and TNF-α polymorphisms • TNFR and IL-1Ra polymorphisms • PD-1 polymorphisms • CTLA-4 polymorphisms • PTPN22 polymorphisms • IRF5 polymorphisms • STAT4 polymorphisms

Category	Marker
Disease diagnosis	• Anti-dsDNA • Anti-ribosomal P protein • Erythrocyte-bound C4d/erythrocyte-CR1 • Platelet-bound C4d
Specific organ involvement	• Renal : Anti-dsDNA / Anti-C1q Antinucleosome /Urinary sVCAM • NP-SLE : Anti-ribosomal P protein Anti-NR2
Disease activity	• Anti-dsDNA, anti-C1q, antinucleosome • Serum complement and activation product levels (C3, C4, C3a, C5a, C3d, C4d, Ba, Bb and sC5b-9) •S. level of IL-6, 10, 12, 15,18, IFN-α, γ, TNF-α) • Soluble IL-2R, TNFR and IL-1Ra • Soluble cell-surface molecules (BLyS, CD27, 154) • Endothelial activation markers (sICAM, sVCAM; thrombomodulin and circulating endothelial cells) • Acute-phase proteins (CRP, ferritin) • Cellular markers (CD27 high plasma cells, reticulocyte-bound C4d)

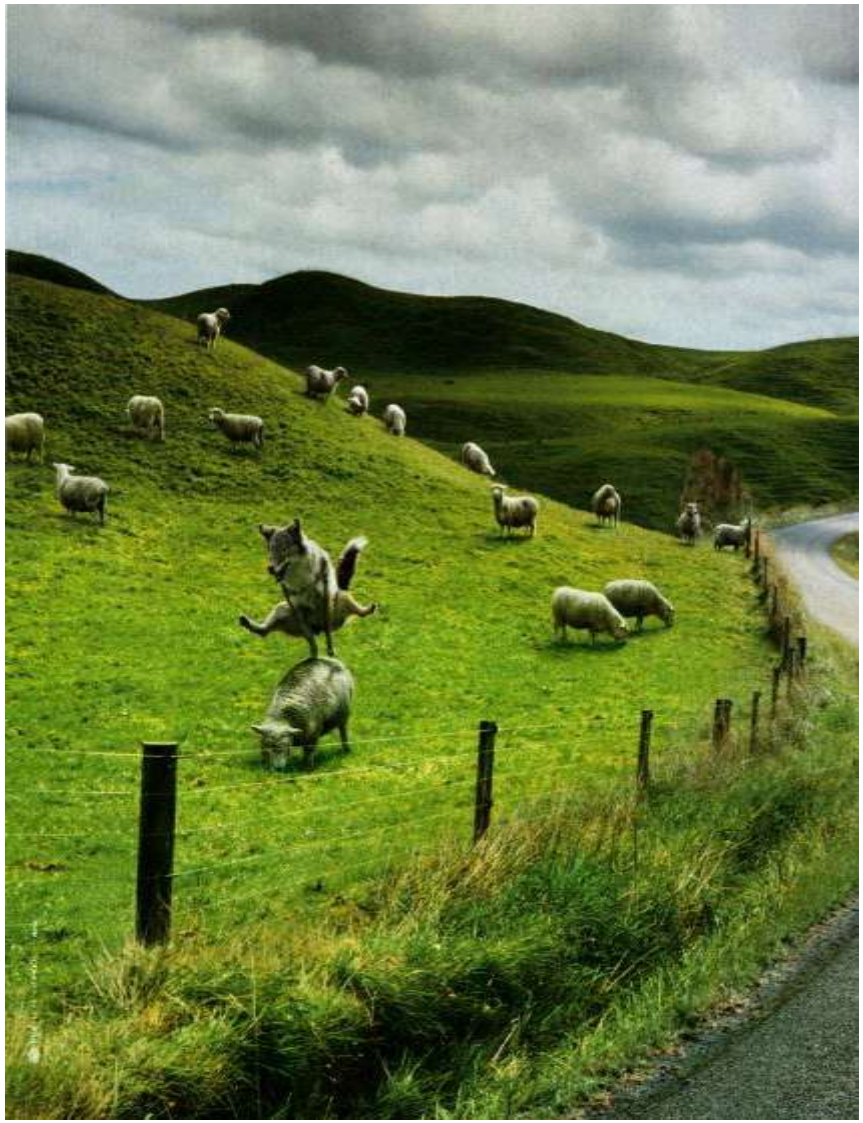
Practice Points

*Many SLE biomarkers in the pipeline are currently "for research use only."

*Measurement of autoantibodies and complement are currently assays of choice in daily clinical practice.

*The results of autoantibody / complement tests are likely most informative if interpreted in a "personalized" manner, i.e., reading the results of each test in the context of the long-term disease course/ manifestations in a given patient.

*Physicians and patients should be educated and encouraged to actively participate in exploratory or validating studies of potential biomarkers.



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2012

THANK YOU